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Institute Report No. 352

Primary Ocular Irritation Potential  
of Ball Powder® in Male Rabbits

Earl W. Morgan, DVM, MAJ, VC  
Gerald F.S. Hiatt, PhD  
and  
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

July 1989

Toxicology Series: 131

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**Primary Ocular Irritation Potential of Ball Powder® in Male Rabbits (Toxicology Series 131)--Morgan *et al.***

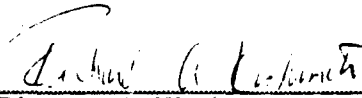
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Richard A. Kishimoto  
COL, MSC  
Acting Commander

20 July 1987  
(date)

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## ABSTRACT

The potential for Ball Powder® to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. Slight conjunctival vasodilation and chemosis (indicative of mild inflammation) and three small pinpoint erosions were the most serious responses observed. The results indicate that Ball Powder® is not a primary ocular irritant under conditions of this study.

Key Words: Ball Powder®, Nitrocellulose, Ocular Irritation, Mammalian Toxicology, Rabbits, Munition, Propellant

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## **PREFACE**

**TYPE REPORT:** Primary Eye Irritation GLP Study Report

**TESTING FACILITY:**

US Army Medical Research and Development Command  
Letterman Army Institute of Research  
Presidio of San Francisco, CA 94129-6800

**SPONSOR:**

US Army Medical Research and Development Command  
US Army Biomedical Research and Development Laboratory  
Fort Detrick, MD 21701-5010  
Project Officer: Gunda Reddy, PhD

**PROJECT/WORK UNIT/APC:** 3E162720A835/180/TLB0

**GLP STUDY NUMBER:** 84037

**STUDY DIRECTOR:** LTC Don W. Korte, Jr., PhD, MSC  
Diplomate, American Board of Toxicology

**PRINCIPAL INVESTIGATOR:** MAJ Earl W. Morgan, DVM, VC, Diplomate  
American College of Veterinary Preventive Medicine  
American Board of Toxicology

**CO-PRINCIPAL INVESTIGATOR:** Gerald F.S. Hiatt, PhD

**REPORT AND DATA MANAGEMENT:**

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

**TEST SUBSTANCE:** Ball Powder®

**INCLUSIVE STUDY DATES:** 24 January 1985 - 5 March 1985

**OBJECTIVE:**

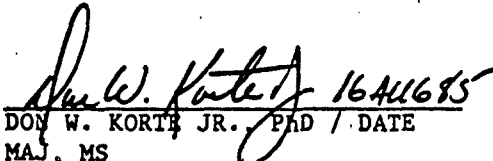
The objective of this study was to determine the primary ocular irritation potential of Ball Powder® in male New Zealand White rabbits.


## **ACKNOWLEDGMENTS**

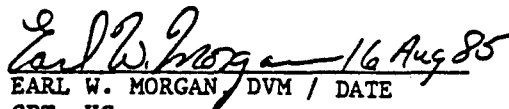
Charlotte Speckman provided technical assistance. SP4 James J. Fisher, PFC Scott L. Schwebe, Richard D. Spieler, Charlotte Speckman, and Diane Arevalo provided care for the animals. Colleen S. Kamiyama and Brenda V. Goce provided administrative and clerical support during the performance of this study and preparation of the report.

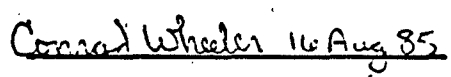
## SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

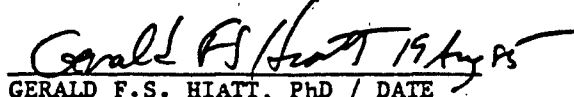
We, the undersigned, declare that GLP Study 84037 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

  
DON W. KORTE JR., PhD / DATE  
MAJ, MS  
Study Director

  
LANCE O. LOLLINI, DVM / DATE  
LTC, VC  
Pathologist

  
EARL W. MORGAN, DVM / DATE  
CPT, VC  
Principal Investigator

  
CONRAD R. WHEELER, PhD / DATE  
DAC  
Analytical Chemist

  
GERALD F.S. HIATT, PhD / DATE  
DAC  
Co-Principal Investigator



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH  
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ATTENTION OF:

SGRD-ULZ-QA

10 July 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84037

1. This is to certify that the protocol for LAIR GLP Study 84037 was reviewed on 1 November 1984.
2. The institute report entitled "Primary Ocular Irritation of Ballpowder," Toxicology Series 131, was audited on 12 May 1987.

*Carolyn M. Lewis*

CAROLYN M. LEWIS, MS  
Diplomate, American Board of  
Toxicology  
Quality Assurance Auditor

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**Primary Ocular Irritation Potential of Ball Powder® in Male Rabbits—  
Morgan et al.**

## **INTRODUCTION**

Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. A genetic and acute mammalian toxicity profile of Ball Powder®, a fielded nitrocellulose-based propellant, was also requested as a baseline against which future formulations will be compared.

### Objective of Study

The objective of this study was to determine the primary ocular irritation potential of Ball Powder® in male New Zealand White rabbits.

## **MATERIALS**

### Test Substance

Name: Ball Powder® (Olin WC 844 double base spheroidal propellant)

LAIR Code Number: TA45

Chemical Composition:

<u>Component</u>	<u>Percent</u>
Nitroglycerin	10.235
Dinitrotoluene	0.685
Diphenylamine	1.105
Dibutylphthalate	5.255
Nitrocellulose	83.23
Total Volatiles	1.045
Moisture and Volatiles	0.895
Residual Solvent	0.49
Calcium Carbonate	0.09
Sodium Sulfate	0.12

Source: Badger Army Ammunition Plant  
Baraboo, WI 53913

Other test substance information is presented in Appendix A.

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 85F026, 85F028 - 85F031, and 85F039. Animal weights on dosing day ranged from 3.0 to 3.9 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dumptanks. The diet consisted of approximately 150 g/day of Certified Purina Chow® Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 17.8°C to 20.6°C and relative humidity ranged from 31% to 58%, except for occasional humidity spikes as high as 65% (room washing). The photoperiod was 12 hours of light per day.

## METHODS

Conduct of this study was in accordance with the LAIR Standard Operating Procedure OP-STX-33, "Primary Eye Irritation Study", and guidelines promulgated by the EPA for ocular irritation testing (2,3).

### Group Assignment/Acclimation

Study rabbits were assigned to two dose groups of 3 males each. These animals were quarantined in the Division of Animal Care and Services for 14 days and acclimated for 12 days in the GLP Suite before dosing. While in quarantine the animals were treated once with Canex® and mineral oil for ear mites. During these periods they were observed daily for signs of illness.

### Dosage Levels and Administration

One-tenth milliliter (0.113 g) of Ball Powder® was administered once to one eye of each rabbit by gently pulling the lower lid away from the conjunctival cul-de-sac to form a cup into which the compound was instilled. Upper and lower lids were then held gently together for one second to prevent loss of material.

### Compound Preparation

Ball Powder® is a spheroidal (0.5 - 1.5 mm) pellet and was administered neat (without any physical modification).

### Test Procedures

On 18 Feb 85, both eyes of each Group 1 animal were examined, for any preexisting abnormalities, by the procedure detailed under the "Ocular Examination/Grading" subheading. For each animal, the eye with the nearest normal appearance was designated for treatment, the other eye serving as an untreated control. On 19 Feb 85, a dose of 0.1 ml Ball Powder® was placed in the designated eye of each rabbit in this group. Group 2 rabbits underwent the same examination on 25 Feb 85 and the same treatment procedure on 26 Feb 85.

#### Ocular Examination/Grading

Initially, each eye was observed unaided in a darkened room with focal illumination (penlight). Structures examined included the lids and surrounding fur, the conjunctiva (semilunar, palpebral, and bulbar), the cornea, and the iris. Grading of the cornea, iris, and conjunctiva was performed according to Table 1 (4). During the 24-, 48-, and 72-hour observations, each eye was also examined with a slit lamp. Special attention was given to integrity of the corneal surface, thickness of the corneal stroma, clarity of anterior chamber fluid, iridial morphology, clarity of the lens, and lenticular surface morphology (5). Additionally, any areas appearing grossly abnormal were examined under high magnification. All observations, including normal appearance, were detailed on the grading sheet. Following this, fluorescein dye (Fluor-I-Strips, Ayerst Laboratories, Inc., New York, NY) was introduced into the eye, which was then observed under ultraviolet light. Any corneal areas reacting with the dye (a sign of discontinuity of the corneal epithelium) were described with respect to area and intensity of fluorescence. Examination and grading of ocular reactions were performed in this fashion at 1, 4, 24, 48, and 72 hours after dosing. Fluorescein staining was omitted from the 1- and 4-hour observations. Due to an almost total lack of reaction on the 7th day after dosing, the study was terminated in accordance with the protocol, and the animals were submitted for necropsy. No scoring or observations were performed at 14 or 21 days.

#### Duration of Study

Appendix C is a complete historical listing of study events.

#### Changes/Deviations

Slit lamp examination was added to the standard observation procedures. The slit lamp enables one to detect subtle reactions not grossly observable and to evaluate more thoroughly those abnormalities which are grossly observable. Color photographic documentation was not performed due to lack of significant response to test compound.

TABLE 1: Grades for Ocular Lesions\*

CORNEA	
<b>Opacity: degree of density (area of greatest density taken for reading)</b>	
No ulceration or opacity .....	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible .....	1†
Easily discernible translucent areas, details of iris slightly obscured .....	2
Nacreous areas, no details of iris visible, size of pupil barely discernible ....	3
Opaque cornea, iris not discernible through opacity.....	4
IRIS	
Normal .....	0
Markedly deepened rugae, congestion, swelling, moderate circumiridial hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive) .....	1†
No reaction to light, hemorrhage, gross destruction (any or all of these) .....	2
CONJUNCTIVA	
<b>Redness: (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)</b>	
Blood vessels normal .....	0
Some blood vessels definitely hyperemic (injected).....	1
Diffuse, crimson color, individual vessels not easily discernible.....	2†
Diffuse, beefy red.....	3
<b>Chemosis: (lids and/or nictitating membranes)</b>	
No swelling .....	0
Any swelling above normal including nictitating membranes.....	1
Obvious swelling with partial eversion of lids.....	2†
Swelling with lids about half-closed.....	3
Swelling with lids more than half-closed.....	4

\* Adapted from Table 6 in Draize et al. (4).

† Indicates minimum level for a positive response.

Group 1 animals were sent to necropsy on 27 Feb 85 instead of 26 Feb as specified in the protocol because of a scheduling conflict in necropsy.

With these exceptions, this study was completed in accordance with the appropriate protocol and addenda. It is believed that none of these changes/deviations had a negative effect on the performance of the study or the validity of the results.

#### Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

### **RESULTS**

Tabulation of the Draize-type ocular grading results is presented in Appendix D and a summary of the ocular observations in Appendix E.

Significant amounts of the test compound were present in the conjunctival cul-de-sac of the six rabbits at one and four hours after dosing. Reduced quantities of the test compound, ranging from a few granules to moderate amounts, were present in the treated eyes of the six rabbits 24 hours after dosing. A few granules of the test compound could still be observed in the eye of one rabbit (85F039) 48 hours after dosing. No test compound was observed in any rabbit's eye 72 hours after dosing.

#### Cornea

Ball Powder<sup>®</sup> produced no grossly observable effects in the cornea. All treated eyes were assigned zero scores for both opacity and area involvement at all observations after dosing.

Slit lamp examination with fluorescein staining revealed small pinpoint corneal erosions in 2 rabbits (85F028, 85F030). These erosions were present at the 24-, 48-, and 72-hour observations. One rabbit (85F039) exhibited a very small corneal erosion on Day 7 after dosing. However, this

rabbit's cornea had been normal until this observation, and since no other lesions were detected in any of the rabbits after 72 hours, this was considered an incidental finding. All other slit lamp observations revealed corneas of normal thickness, indicating lack of edema, and smooth surfaces, indicating epithelial integrity.

#### Iris/Anterior Chamber

No grossly observable reactions were produced in the iris by Ball Powder®. Iridial scores were consistently zero at all observation times.

One rabbit (85F026) on slit lamp examination exhibited very slight increased vascularization of circumiridial vessels at the 24-hour observation. No other iridial abnormalities were detected by slit lamp examination of the treated eyes. Circumiridial vessels (with the one exception) and surface morphology were normal at all times after dosing. Close examination of anterior chamber fluid revealed no evidence of the presence of protein or cells (signs of iridial inflammation).

#### Lens

The lens was not scored under the Draize-type grading system because of the difficulty in making unaided observations. At all times after dosing, the lens appeared normal during slit lamp examination. No changes were observed in clarity or surface morphology.

#### Conjunctiva

In this study, Ball Powder® produced only two grossly observable responses—slight conjunctival redness and swelling. At 1 hour after dosing, 2 of 6 treated eyes exhibited slight vasodilatation in the bulbar (sclera) or semilunar (nictitating membrane) conjunctiva. At 4 hours after dosing, all 6 treated eyes exhibited slight vasodilatation. The vasodilatation decreased to 4 of 6 at 24 hours and only 1 of 6 at 48 hours after dosing. Conjunctival redness scores of 1 were assigned to the treated eyes and slit lamp examination confirmed the presence of dilated vessels within the outer layers

of the sclera and the nictitating membrane. At 1 hour, one animal, and at 4 hours, three animals exhibited slight conjunctival swelling, graded 1 on the Draize scale. Swelling of the nictitating membrane was confirmed by slit lamp examination in these rabbits.

#### Control Eyes

At no time during the study did the untreated eyes exhibit any change from their normal condition on the day of dosing. Small corneal lesions were observed in four of the control eyes at the preliminary examination 24 hours before dosing. These slight lesions resolved by the day of dosing and no further abnormalities were observed during the study.

#### Pathology Report

Lesions observed were considered incidental and in no way related to the treatment. The pathologist's report is presented in Appendix F.

### **DISCUSSION**

The primary goal of ocular toxicity testing is to determine the potential for ocular damage resulting from accidental contact of the test compound with the eye. For this purpose, the Draize-type irritation test, used in the present study, is especially well-suited. An important feature of this test is that the route and type of exposure (ocular instillation followed by a forced blink) closely mimics potential human exposures.

Consumer Product Safety Commission Guidelines, which the EPA recommends for ocular irritation testing, state that an animal has exhibited a positive reaction if the test substance produces one or more of the following signs: ulceration of the cornea (other than a fine stippling); opacity of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than a slight deepening of the rugae or a slight hyperemia of the circumcorneal blood vessels); an obvious swelling in the conjunctiva with partial eversion of the lids; or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible (2).

Guidelines for classification of chemicals as ocular irritants or nonirritants have been published and form the basis for evaluation in the present study (6). These Interagency Regulatory Liaison Group (IRLG) guidelines state: "[a] test result is considered positive if four or more animals exhibit a positive reaction. If only one animal exhibits a positive reaction, the test result is regarded as negative."

In this study, Ball Powder® produced no positive reactions, as defined by the IRLG. Slight conjunctival redness and swelling, indicating mild inflammation, and three small pinpoint erosions were the only responses observed. Since Ball Powder® is insoluble in physiological solutions, these minor reactions could be attributed to physical irritation. These reactions, although scorable, did not achieve sufficient severity to warrant consideration as a "positive response." Due to this lack of positive response, Ball Powder® is classified as a nonirritant by the results of the present study.

#### **CONCLUSION**

Ball Powder® exhibited minimal potential to produce ocular irritation under conditions of this study.

## REFERENCES

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## Appendix A: CHEMICAL DATA

**Appendix B: ANIMAL DATA**

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry  
5265 Starr Way  
Watsonville, CA 95076

Sex: Male

Age: Young adults

Animals in each group: 3 males

Condition of animals at start of study: Normal

Body weight range at dosing: 3.0 - 3.9 kg

Identification procedures:

Ear tattoo numbers 85F026, 85F028 - 85F031, 85F039.

Pretest conditioning:

1. Quarantine/acclimation from 24 Jan - 18 Feb 1985
2. Animal eyes were examined 24 hours before dosing using slit lamp, fluorescein dye, and ultraviolet light.

Justification:

Laboratory rabbits are a proven sensitive animal model for ocular testing.

### Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
24 Jan 85	Animals arrived at LAIR.
25 Jan 85	Animals were tattooed, weighed, examined for illness, placed under a two-week quarantine, and given one application of Canex®/mineral oil.
25 Jan - 7 Feb 85	Animals were checked daily by quarantine personnel.
7 Feb 85	Rabbits were certified healthy by a staff veterinarian and moved from quarantine to the GLP Suite.
8 Feb 85	Rabbits were separated into test groups and weighed.
18 Feb 85	Animals were checked for preexisting ocular injury (Group 1).
19 Feb 85	Group 1 rabbits were dosed and weighed. Eyes were scored 1 and 4 hours after exposure.
20 Feb 85	Eyes were scored 24 hours after exposure (Group 1).
21 Feb 85	Eyes were scored 48 hours after exposure (Group 1).
22 Feb 85	Eyes were scored 72 hours after exposure (Group 1).
25 Feb 85	Animals were checked for preexisting ocular injury (Group 2).
26 Feb 85	Eyes were scored 7 days after exposure (Group 1). Study of Group 1 was terminated and animals were weighed. Group 2 rabbits were dosed and weighed. Eyes were scored 1 and 4 hours after exposure.
27 Feb 85	Group 1 animals were submitted to necropsy. Eyes were scored 24 hours after exposure (Group 2).
28 Feb 85	Eyes were scored 48 hours after exposure (Group 2).
1 Mar 85	Eyes were scored 72 hours after exposure (Group 2).
5 Mar 85	Eyes were scored 7 days after exposure (Group 2). Study (Group 2) was terminated and animals were weighed and submitted for necropsy.

## Appendix D: TABULATED OCULAR DATA

CORNEAL OPACITY  
(score by animal)

<u>Rabbit Number</u>	<u>Base- Line</u>	<u>1 hr</u>	<u>4 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>
85F026	0	0	0	0	0	0
85F028	0	0	0	0	0	0
85F029	0	0	0	0	0	0
85F030	0	0	0	0	0	0
85F031	0	0	0	0	0	0
85F039	0	0	0	0	0	0

IRIS  
(score by animal)

<u>Rabbit Number</u>	<u>Base- Line</u>	<u>1 hr</u>	<u>4 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>
85F026	0	0	0	0	0	0
85F028	0	0	0	0	0	0
85F029	0	0	0	0	0	0
85F030	0	0	0	0	0	0
85F031	0	0	0	0	0	0
85F039	0	0	0	0	0	0

## Appendix D (cont.): TABULATED OCULAR DATA

## CONJUNCTIVA (CHEMOSIS)

(score by animal)

<u>Rabbit Number</u>	<u>Base- Line</u>	<u>1 hr</u>	<u>4 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>
85F026	0	1	1	0	0	0
85F028	0	0	1	0	0	0
85F029	0	0	0	0	0	0
85F030	0	0	1	0	0	0
85F031	0	0	0	0	0	0
85F039	0	0	0	0	0	0

## CONJUNCTIVA (REDNESS)

(score by animal)

<u>Rabbit Number</u>	<u>Base- Line</u>	<u>1 hr</u>	<u>4 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>72 hr</u>
85F026	0	1	1	1	0	0
85F028	0	0	1	1	0	0
85F029	0	1	1	1	0	0
85F030	0	0	1	0	1	0
85F031	0	0	1	0	0	0
85F039	0	0	1	1	0	0

## **Appendix E: SUMMARY OF OCULAR OBSERVATIONS**

### One Hour After Dosing

Slight hyperemia was present in 2 of the 6 test rabbits. This hyperemia was confined to the lower bulbar and palpebral conjunctiva and the nictitating membrane. Slight swelling (chemosis) of the nictitating membrane was also present in one rabbit. Both the vasodilatation and chemosis were visible with the unaided eye. All other structures appeared normal.

### Four Hours After Dosing

Slight hyperemia was present in the conjunctiva of all rabbits. Slight conjunctival chemosis was present in 3 of 6 rabbits. All other structures appeared normal.

### Twenty-four Hours After Dosing:

Slight hyperemia persisted in 4 of 6 rabbits. Small corneal erosions were noted in 2 rabbits (85F028, 85F030) after fluorescein staining. On slit lamp examination all other structures appeared normal with the exception of 3 animals that had very slight edema of the papillae along the margin of the nictitating membrane and medial canthus.

### Forty-eight Hours After Dosing

Slight hyperemia was present in the conjunctiva in 1 of 6 rabbits. Pinpoint corneal erosions were still present in 2 rabbits. All other structures in each treated eye appeared normal, even by slit lamp examination.

### Seventy-two Hours After Dosing

Pinpoint corneal erosions were still present in the 2 rabbits. All other structures examined by slit lamp appeared normal.

### Seven Days After Dosing

A pinpoint corneal erosion was noted in rabbit 85F039. All other structures examined by slit lamp appeared normal.

## Appendix F: PATHOLOGY REPORT

### LAIR Gross Pathology Report GLP Study J4837

Study: GLP #84837, Toxicology Services Group

Test: Primary Ocular Irritation

Investigator: CPT Morgan

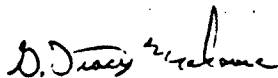
Test Substance: Ball powder (OLIN WC 844 double-base spheroidal propellant)

History: Study conducted in accordance with SOP-OP-STX-33. Number of animals: 6. Sex: male, Species: Rabbit NZW.

#### Findings:

<u>Animal ID #</u>	<u>LAIR Path #</u>	<u>Lesions</u>
85F026	36963	1. Pinworms - cecum 2. white focus (3mm), liver
85F028	36964	None
85F030	36965	White foci #8 (1-3mm), liver
85F029	37011	Pinworms - cecum
85F031	37012	Pinworms - cecum
85F039	37013	None

Comments: The lesions noted were considered incidental and not related to the treatment.



G. TRACY MARKOVEC, DVM  
CPT, VC  
Pathology Services Group



LANCE O. LOLLINI, DVM  
LTC, VC  
Chief, Pathology Services Group

## Distribution List

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Alexandria, VA 22333

**HQDA**

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Falls Church, VA 22041-3258

**HQDA**

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20 Massachusetts, NW  
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**CDR, US Army Toxic and Hazardous  
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United States Army  
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